

What is claimed is:

1. A method for determining the amount of pyruvate dehydrogenase (PDH) complex in a biological sample comprising:
 - a) contacting a sample comprising PDH complex with an isolated antibody that specifically binds to PDH complex under conditions to allow specific binding of the antibody to solubilized PDH complex present in the sample to form an immunocomplex;
 - b) separating remaining sample contents from the immunocomplex; and
 - c) detecting the amount of the PDH complex in the separated immunocomplex, thereby determining the amount of the PDH complex in the patient sample.
2. The method of claim 1, wherein the antibody is an anti-E2 specific antibody.
3. The method of claim 1, wherein the antibody is a monoclonal antibody.
4. The method of claim 1, wherein the PDH complex is obtained from a cell.
5. The method of claim 1, wherein the sample is obtained from solubilized human fibroblasts or human heart mitochondria.
6. The method of claim 1, wherein the PDH complex in the immunocomplex retains PDH activity.
7. The method of claim 1, wherein the antibody is attached to a solid support and the separating includes separating unbound sample contents from the solid support.

8. The method of claim 7, wherein the separating in b) comprises:
 - (i) releasing the immunocomplex complex; and
 - (ii) separating the immunocomplex from other components of the sample using SDS-PAGE.
9. The method of claim 7, wherein the detecting in c) comprises contacting immunocomplexed PDH complex with a detectable marker that binds specifically to the immunocomplexed PDH and measuring the amount of detectable marker present on the solid support.
10. The method of claim 7, wherein the solid support is a microtiter plate or beads.
11. The method claim 10, wherein the detecting in c) comprises high throughput screening.
12. The method of claim 1, wherein the sample is derived from a patient in need of diagnosis of a PDH complex-associated disease.
13. The method of claim 1 further comprising quantifying the immunocaptured PDH complex detected in the sample by comparing with a standard reference curve obtained using a purified sample of PDH complex.
14. The method of claim 13, further comprising determining specific activity of the immunocaptured PDH complex.
15. The method of claim 14, wherein the sample is obtained from a patient sample and wherein the method further comprises distinguishing between a defect in PDH complex turnover rate and a defect in production of PDH complex in the patient.

16. A method for measuring activity of PDH complex in a sample, said method comprising:
- a) contacting a sample comprising PDH complex with an isolated antibody that specifically bind to PDH complex with under conditions to allow formation of an immunocomplex of the antibody and the PDH complex present in the sample;
 - b) contacting the immunocomplex with a reaction mixture comprising a non-limiting amount of one or more substrates necessary for activity of the PDH complex; and
 - c) detecting the amount of NADH produced in the reaction mixture, wherein the amount of NADH produced indicates the active state of the PDH complex.
17. The method of claim 16, wherein the PDH complex is derived from solubilized human fibroblasts, human heart mitochondria or bovine heart mitochondria.
18. The method of claim 16, wherein the substrates are β -NAD⁺, Coenzyme A, FAD⁺, cysteine, pyruvate, and thiamine pyrophosphate (TPP).
19. The method of claim 16, wherein the detecting in c) comprises:
- (i) transferring an electron from reduced NADH to an electron acceptor molecule to produce NADH; and
 - (ii) determining a change indicating transfer of an electron to the electron acceptor molecule, wherein magnitude of the change indicates biological activity of the PDH complex.

20. The method of claim 19, wherein the electron acceptor molecule is an electron acceptor dye molecule; and

the determining in (ii) involves monitoring the reaction mixture spectrophotometrically to determine a change in absorbance of the electron acceptor dye molecule; wherein magnitude of the change indicates biological activity of the PDH complex as compared to that of a comparable healthy sample of PDH complex.

21. The method of claim 20, wherein the electron acceptor dye molecule is selected from diaphorase, resazurin, and a combination thereof.

22. The method of claim 20, wherein the monitoring comprises detecting a change in fluorescence from the dye molecule.

23. The method of claim 20, wherein the detecting in c) comprises contacting the reaction mixture with a PDH inhibitor and comparing an amount of resultant inhibition of the PDH complex compared to that of a comparable healthy sample of PDH complex.

24. The method of claim 23, wherein the PDH complex inhibitor is selected from sodium arsenite and ATP.

25. The method of claim 20, wherein the detecting in c) comprises contacting the reaction mixture with a PDH complex activator and comparing an amount of resultant activation of the PDH complex compared to that of a comparable healthy sample of PDH complex.

26. The method of claim 25, wherein the activator is dichloroacetate.

27. A kit for assaying PDH complex activity in a sample comprising an antibody specific for said PDH complex

28. The kit of claim 27 further comprising a detectable label for the antibody.
29. A method for determining the level of activity in PDH complex in a sample, said method comprising:
- a) contacting an isolated antibody that specifically binds to PDH complex with a sample comprising PDH complex under conditions that allow specific binding of the antibody to PDH complex present in the sample to form an immunocomplex;
 - b) contacting the immunocomplex with a reaction mixture comprising a non-limiting amount of one or more substrates necessary for activity of the PDH complex;
 - c) separating remaining sample contents from the immunocomplex; and
 - d) detecting the level of phosphorylation of immunocomplexed PDH complex in the in the sample as compared with that of an unphosphorylated PDH complex standard, wherein a level of phosphorylation greater than that in the standard indicates a lowered level of activity, and a level of phosphorylation substantially equal to that of the PDH complex in the sample indicates a normal level of activity of the PDH complex in the sample.
30. The method of claim 29, wherein the level of phosphorylation is compared by measuring an amount of negative isoelectric point shift of the immunocomplexed PDH complex compared to the isoelectric point of the standard, the amount of negative isoelectric point shift being directly proportional to the amount of phosphorylation of the PDH complex in the sample.
31. The method of claim 30, wherein the sample is derived from a patient and wherein the amount of negative isoelectric shift is used to screen the patient for a disorder of PDH complex activity.
32. The method of claim 31, wherein the disorder is a disorder of energy production or utilization.

33. The method of claim 32, wherein the disorder is diabetes.
34. A method for screening to detect an active agent that modifies inhibitor or activator activity of a known inhibitor or activator of PDH complex comprising:
- a) contacting a sample containing PDH complex in the presence of a known inhibitor or activator and a test active agent with a PDH complex immunoprecipitating antibody under conditions that allow formation of an antibody/PDH complex immunocomplex; and
 - b) determining the degree to which the test active agent modifies the inhibitor or activator activity of the known inhibitor or activator in the sample as compared to inhibitor or activator activity of the known inhibitor or activator in the absence of the test active agent, thereby detecting an active agent that modified inhibitor or activator activity of a known inhibitor or activator of PDH complex.
35. The method of claim 34, wherein the active agent is selected from a small molecule, a drug, or a protein.
36. The method of claim 34, wherein the PDH complex inhibitor is sodium arsenite or ATP and the test active agent decreases inhibitor activity of the PDH complex inhibitor.
37. The method of claim 34, wherein the PDH complex activator is dichloroacetate and the test active agent decreases activator activity of the PDH complex activator.
38. The method of claim 34, wherein the antibody is an anti-E2 specific antibody.
39. The method of claim 34, wherein the antibody is a monoclonal antibody.

40. The method of claim 32, wherein the PDH complex is derived from solubilized human fibroblasts or human heart mitochondria.

41. A method for screening patients to identify patients suspected of having a late onset mitochondrial disorder, said method comprising:

- a) contacting isolated antibodies that immunoprecipitate PDH complex with a patient sample comprising solubilized PDH complex so that the antibodies bind to solubilized PDH complex present in the sample to form an immunocomplex;
- b) separating the immunocomplex from the remaining sample contents; and
- c) detecting a decrease in the amount of PDH complex as compared with an amount in a corresponding normal sample, wherein the decrease indicates the patient is suspected of having the late onset mitochondrial disorder.

42. The method of claim 41, wherein the late onset mitochondrial disorder is selected from late onset diabetes, Huntington's, Parkinson's and Alzheimer's diseases, ALS (amyotrophic lateral sclerosis), and Schizophrenia.

43. The method of claim 41, wherein the separating in b) comprises:

- a) releasing the immunocomplex; and
- b) separating the immunocomplex from other components of the sample using SDS-PAGE.

44. The method of claim 41, wherein the anti-PDH complex antibodies are attached to a solid support and the antibodies are tagged with a detectable marker.

45. The method of claim 44, wherein the detecting in c) comprises contacting the immunocomplex with a detectable marker that binds specifically to the immunocomplex and measuring the amount of signal from the detectable marker present on the solid support.

46. The method of claim 44, wherein the solid support is beads.

47. The method of claim 44, wherein the solid support is a micro-titer plate.

48. The method claim 44, wherein the detecting in c) comprises high throughput screening